Claims 1-21 are pending in the application.

To begin, the Office maintains the rejection of Claims 1-16 for failing to comply with the written description requirement under 35 U.S.C. § 112, first paragraph. It is the position of the Office that the Specification lacks a definition or description of the structural characteristics commonly possessed by TnaA so that one skilled in the art would recognize that TnaA sequences lacking tryptophanase activity were in the possession of the Applicants. The Office suggests that any sequence introduced into a cell which inhibits indole production falls within the broad genus of sequences lacking tryptophanase activity.

The instant invention is drawn to a sophisticated expression system for tightly regulating the expression of recombinant proteins. An expression cell line, having a mutation in the *tna*A locus of the host cell genome, was created as a result of homologous recombination and integration of heterologous DNA within the *tna*A locus of the host genome which result is an inactivation of the expression of the gene product, tyrptophanase. The absence of tryptophanase activity, the discernable phenotype of the gene knock-out, was determined using the disclosed indole test. Tryptophanase is an enzyme that catalyzes the degradation of tryptophan to indole, pyruvate and ammonia. The indole test is a standard assay to measure the amount of indole which is converted from tryptophan by the enzymatic activity of tryptophanase. The lack of enzymatic activity as demonstrated using this assay is confirmatory for the phenotype, absence of indole, which results from tryptophanase gene knockout. The strategy for determining that the host cell *tna*A gene has been inactivated is described in the instant Specification on page 18. The integration of the instant defective *tna*A gene into the host chromosome is first verified by genomic analysis, i.e., PCR amplification. The loss of enzymatic activity of the host tryptophanase, as a result of the integrated inactivating gene is subsequently assayed using the indole test to confirm the genetic manipulation.

Thus, the Office conclusion that any mutant cell that does not produce indole would fall within the broad scope of the claim is not accurate. It is not true that tryptophanase knock-out is the only possible cause of indole absence. The absence of indole could be caused by any number of metabolic flaws in normal host function. The indole test is simply a crude evaluation of the desired phenotype following genomic analysis to confirm integration at the targeted *tna*A locus. The Applicants submit that the teaching of the Specification and the claimed limitation to the integration of a nucleic acid sequence which is capable of inactivating the host gene encoding TnaA tryptophanase provide sufficient written description for the claimed invention.

The Office asserts that a sequence encoding any protein, which results in inhibition of indole production, falls within the broad genus of sequences lacking tryptophanase activity. The Applicants traverse this conclusion and submit that the genus of sequences which abolish tryptophanase activity is limited to those sequences which recombine and integrate within the genomic *tna*A locus of a transformed cell to abolish, or knock-out, expression of endogenous tryptophanase activity. The instant invention is drawn to an expression system enhanced by the repression of endogenous tryptophanase activity. As explicitly stated in Claim 1 (a), a prokaryotic cell is transformed with a vector containing a nucleic acid sequence which inactivates the gene encoding TnaA in the host cell. The language of the claim specifically states that the nucleic acid integrates into the DNA of the host cell. Inclusion of this language is limiting to those specific cells in which the endogenous gene for *tna*A has been replaced, i.e., knocked-out, through homologous recombination and integration of heterologous nucleic acid sequence which does not encode a functional tryptophanase protein, not as the Office would suggest just any protein which results in inhibition of indole production. The Applicants submit that the instant Specification provides positive definition of claimed subject matter and thereby, obviates the U.S.C § 112, first paragraph rejection for lack of written description.

The Office requires examples of common structural characteristics that correlate with intended function, namely the inactivation of tryptophanase activity. The gene encoding tryptophanase is well-defined and is described in the reference to Deeley, et al., as cited in the instant Specification. The Applicants submit herewith, a reference, Phillips, et al. (Journal of Biochemistry 1989, 264:10627-32), which discloses active site residues, and reference to Kamath et al., (Journal of Biochemistry 1992, 267:19978-85), which discusses key amino acid residues which are relevant for the activity of the tryptophanase enzyme. The references provide guidance as to functionally important amino acid residues which, when knocked-out, would result in a non-functional tryptophanase and provide guidance as to nucleic acid sequences which may be inactivating. The references substantiate the level of skill in the art regarding nucleic acid sequences which do not encode the required active site catalytic residues, substrate binding residues and residues for co-factor binding which, when integrated into the host *tna*A locus, would not express an active tryptophanase.

Through genomic analysis, the Applicants have demonstrated the integration of the defective gene into the host genome to abolish expression and thus, activity of the endogenous tryptophanase enzyme. The Applicants have demonstrated enhanced performance characteristics of the *tna*A gene knock-out by the disclosure of ICONE-100 and ICONE-200. The Applicants have demonstrated the resultant phenotype of the gene knock-out as evidenced by the absence of tryptophanase activity in the recombinant cells using the standard indole test.

The instant expression system for the tightly controlled regulation of recombinant protein expression is based on the well-defined genetic mutations which are controlled genetically and biochemically and are targeted to the *tna*A locus of *E. coli* without the necessity for a selection marker. The methodology for the construction of the recombinant cell line is described in detail in the aforementioned Examples such that one of skill in the art would conclude that the Applicants were in possession of the necessary common attributes possessed by

the members of the genus. In light of these remarks, the Applicants submit that compliance with the written description requirement is met. Reconsideration of the rejection, and withdrawal thereof, is respectfully submitted.

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The Applicants hereby submit an Information Disclosure Statement, Form PTO-1449, to comply with 37 CFR § 1.98(a)(1). As will be noted, this Information Disclosure Statement calls a number of references, which might be considered relevant, to the attention of the Office. The fact that these are in fact "prior art" and/or relevant to the prosecution is, however, not admitted.

* * * * *

Accordingly, reconsideration of all grounds of objection and rejection, withdrawal thereof, and passage of this application to issue are all hereby respectfully solicited.

It should be apparent that the undersigned attorney has made an earnest effort to place this application into condition for immediate allowance. If he can be of assistance to the Examiner in the elimination of any possibly-outstanding insignificant impediment to an immediate allowance, the Examiner is respectfully invited to call him at his below-listed number for such purpose.

Allowance is solicited.

Respectfully submitted,

THE FIRM OF HUESCHEN AND SAGE

By: 

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Enclosure: Information Disclosure Statement, Form PTO 1449; References: Kamath, et al., Phillips, et al. and Postal Card Receipt

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THE COMMISSIONER IS HEREBY AUTHORIZED TO CHARGE ANY FURTHER OR ADDITIONAL FEES WHICH MAY BE REQUIRED (DUE TO OMISSION, DEFICIENCY, OR OTHERWISE), OR TO CREDIT ANY OVERPAYMENT, TO DEPOSIT ACCOUNT NO. 08,3220.